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THE OSMOTIC AND SURFACE TENSION PHENOMENA OF LIVING ELEMENTS AND THEIR PHYSIOLOGICAL SIGNIFICANCE.[1]

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CONTENTS.

PREFACE.

This paper formed the basis for two lectures given before the class in physiology at Woods Hole, July 7 and 8, 1911, although owing to limited time, some parts were omitted. Since then there has appeared a second edition of Höber's "Physikalische Chemie der Zelle und Gewebe," which reviews much of the literature considered in this paper. However, owing to an entirely different mode of presentation, it is hoped that the present treatment of the subject might be helpful to many general readers, some of whom would not read Höber's book.

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I am indebted to several persons for suggestions, especially to Dr. Ralph Lillie[1] and Professor B. M. Duggar.

I. INTRODUCTION.

The object of this paper is to bring the "vital" phenomena, as far as possible, within the scope of physics and chemistry, and not to elucidate physical and chemical processes. It should therefore be borne in mind that the osmotic phenomena of "dead" systems are not all satisfactorily explained.

The Vant Hoff-Arrhenius theory of osmosis concerns itself with the number of particles, molecules and ions, in solution, and is applicable to dilute solutions, in which the total volume of the dissolved particles is negligible. However, in more concentrated solutions, the volume of the dissolved particles is of the same importance as the volume of the molecules in gases, as expressed in Van der Waal's equation. Also the dissolved particles bind molecules of the solvent and so reduce the volume of the free solvent.

That the molecules and ions of a dissolved substance bind some molecules of the solvent, follows from the work of Jones and his collaborators.[2] Compare also the work of Pickering.[3] Jones concludes that the larger the number of molecules of water of crystallization, the greater the hydrating power of a substance in aqueous solution. The number of molecules of water bound by one molecule of the solute usually increases with dilution up to a certain point (the boundary between concentrated and dilute solutions, beyond which there is no heat of dilution). The bond between ions and the solvent is also indicated by the phenomenon known as "electrical transference." If an electrolyte and a non-electrolyte be dissolved in water and an electric current passed through the solution, water will be carried along with the ions to the electrodes.

With these corrections, the Vant Hoff-Arrhenius theory accounts for osmotic pressure, but does not show why many substances exert no osmotic pressure, in other words, why no

[1] Cf. this journal, 1909, XVII., 188.

[2] "Hydrates in Aqueous Solution," Pub. No. 8, Carnegie Ins. Wash., 1907.

[3] Whetam, "The Theory of Solution," 1902, Cambridge, p. 170.

membranes have been found that are impermeable to them. Overton supposed that the substance, in order to diffuse, must dissolve in the membrane. Kahlenberg and others consider a solution as a chemical combination between solute and solvent, and osmosis as a series of chemical reactions between the membrane and the two solutions, continuing until equilibrium is established. The essential points in the theory are: that the membrane is not a molecule sieve, but a substance with specific properties, and the chemical characters of the membrane and of the dissolved substances affect osmosis.

Willard Gibbs found that the more a solute lowers the surface tension of a solution, the more it tends to pass out of the solution, *i. e.*, by osmosis, or if this is prevented, to collect at the surface of the solution. This law has been extensively investigated and confirmed by I. Traube. For instance, in general, lipoid-soluble substances lower the surface tension of water and tend to diffuse out of it, whereas electrolytes slightly raise the surface tension of water and attract water from the adjacent phase. Osmosis may occur in opposite directions simultaneously. Gibbs and Traube state that the greatest osmotic flow is from the solution of lower surface tension to that of the higher, but this is not generally accepted. Osmosis consists of two distinct processes, from one solution to the membrane, and from the membrane to the second solution.

In case the membrane consists of two or more chemically different membranes placed one on another, osmosis consists of a series of steps; and Hamburger[1] made double membranes through which certain substances diffuse more rapidly in one direction than in the other.

Traube calls the bond between solute and solvent the "attraction pressure." In general, attraction pressure of ions increases with valence. The less the attraction pressure of the solute, the more it lowers the surface tension and tends to pass out of the solution. The presence of one solute lowers the attraction pressure of another in the same solution, and the greater the attraction pressure of a solute the more it lowers that of another. We might express this idea by saying that one substance takes

[1] *Biochem. Zeit.*, 1908, XI., 443.

part of the solvent away from the second and increases the concentration of the second substance. This may explain the effect of a harmless substance in increasing the toxicity of a poison. Schnerlen[1] observed that a solution of phenol below the threshold of toxicity for certain bacteria is rendered toxic by adding NaCl. Stockard showed that the toxicity of pure solutions of salts on fish eggs is increased by the addition of sugar, although the total osmotic pressure of the mixture is less than that of the normal medium.[2]

Just as Traube's precipitation membranes are absolutely impermeable to certain substances, so do living cells show this selective permeability. For instance, the vacuole fluid or cell sap of certain plant cells contains colored substances which do not diffuse into the protoplasm surrounding the vacuoles. If a cell be placed in a solution of the pigment, the protoplasm remains colorless. If the protoplasm be squeezed out of the cell into a solution of the pigment, it does not invariably become stained. However, if the cell is injured in certain ways, or dies from any cause, the pigment diffuses out of the vacuoles into the protoplasm and thence into the surrounding medium. We might conclude that the protoplasm in general is impermeable to the color, but at death it becomes permeable. On the other hand, Pfeffer[3] gives evidence for the existence of a mechanical membrane on the surface of the cell and lining the vacuoles. De Vries[4] placed cells into 10 per cent. KNO_3 solution colored with eosin. The plasma membrane and granular plasm died and stained long before any dye entered the vacuoles. However, the granular plasm may have absorbed all the dye, thus preventing its entrance for some time, without the necessity of any resistance of the vacuole membrane. Since protoplasm may be squeezed out in the form of droplets and still appears to be surrounded by membranes, Pfeffer concluded that the membrane was formed by the contact of the protoplasm with the medium

[1] *Arch. exp. Path.*, 1896, XXXVII., 84.

[2] However the NaCl in Schnerlen's and sugar in Stockard's experiment may have increased the permeability to the toxic substances, as discussed in later chapters.

[3] "Pflanzenphysiologie."

[4] *Jahrb. wiss. Bot.*, 1885, XVI., 465.

or with cell sap. He supposed these membranes to be the semipermeable parts of the cell, and that they became altered at death. Pfeffer called this membrane on the cell surface the "plasma membrane."

Whereas the nuclear membrane and certain vacuole membranes are semipermeable, these are lacking in erythrocytes, which are therefore good objects for testing the question whether the protoplasm in general, or merely its surface, is semipermeable. Höber[1] by two very ingenious but complicated methods, one based on dielectric capacity, determined the electric conductivity of the interior of the erythrocyte without rupture of the plasma membrane. Since the conductivity of the interior (about that of a .2 per cent. NaCl solution) was found to be many times greater than that of the erythrocyte as a whole, the membrane must be relatively impermeable to ions. There is much other, but less direct, evidence that the semipermeability resides in the plasma membrane, namely: the rapidity of change in permeability of certain cells, the sudden increase in permeability of a cell after swelling to a certain size (due presumably to rupture of the plasma membrane), the ease with which mild mechanical treatment increases the permeability, and the localization of electric polarization at the cell surface.

Quincke[2] supposed these membranes to be of a fatty nature. This idea was carried further by Overton, who considered the plasma membrane to be composed, not of neutral fats, but of substances of the class which are called "lipoids," which included non-saponifying ether soluble extracts of organs, *i. e.*, cholesterin, lecithin, cuorin, and cerebrin. He found[3] that all basic dyes were easily absorbed by living cells, but not most of the sulphonic acid dyes. This corresponded to their solubility in melted cholesterin, or solutions of lecithin and cholesterin, or particles of lecithin, protagon or cerebrin. His argument is somewhat weakened, however, by the fact that cholesterin decomposes on melting, and that if lecithin is allowed to absorb water its solvent power changes.

[1] *Arch. f. d. ges. Physiol.*, 1910, CXXXIII., 237, and Eighth Internat. Physiol. Congress, Vienna, 1910.

[2] *Sitzber. d. Kon. Preuss. Akad. d. Wissensch. zu Berlin*, 1888, Bd. XXXIV.

[3] *Jahrb. wiss. Bot.*, 1900, XXXIV., 669.

Many of Overton's critics do not distinguish between lipoids proper and a host of ether-soluble substances which are also called lipoids, and of the data which they present we will consider only that on lipoids proper. Ruhland[1] found that certain dyes stain plant cells but are not soluble in solutions of cholesterin (and vice versa). Robertson[2] observed that methyl green freed from methyl violet was insoluble in a nearly saturated solution of lecithin in benzol, whereas it stained living cells. Höber[3] obtained Ruhland's results, when using certain animal cells, but found that certain nephric tubule cells absorb all dyes that are not suspension colloids.

Faure-Fremiet, Mayer and Schaeffer[4] state that pure cholesterin does not stain with any dyes (contrary to Overton), malachite green (considered lipoid-insoluble by Ruhland and Höber) stains lecithin, and Bismarck brown (considered lipoid-insoluble by Ruhland) stains lecithin, cholesterin-oleate and cerebrin. A mere trace of free fatty acid greatly affects the behavior of lipoids toward stains.

Mathews[5] considers the absorption of dyes by cells as a chemical process. Since basic dyes combine with albumin in alkaline solution, lipoids in the membrane are not necessary for the absorption of such dyes.

Traube objected to Overton's hypothesis on the ground that Overton's plasmolytic series is the same as found by Brown, who used the membrane of the barley grain,[6] and the same as the series of the attraction pressures of the substances in water. But Traube admits in his later papers that the chemical character of the membrane affects osmosis.

We may conclude that, although the plasma membrane of some cells may be lipoid in character, this has not been proven, but, in general, it is more permeable the more the diffusing substance lowers the surface tension of water.

[1] *Jahrb. wiss. Bot.*, 1908, XLVI., 1, and *Ber. Deutsch. bot. Gesellsch.*, 1909, XXVI., 772.

[2] *Jour. Bio. Chem.*, 1908, LV., 1.

[3] *Biochem. Zeit.*, 1909, XX., 55.

[4] *Arch. d'Anat. Mic.*, 1910, XII., 19.

[5] *Jour. Pharmacol. and Exp. Ther.*, 1910, II., 201.

[6] But this is not true of all seed coats. Atkins, *Sci. Proc. Roy. Dublin Soc.*, XII., n. s., No. 4, p. 35, observed that the membranes of the bean seed are freely permeable, semipermeable plasma membranes arising only after germination.

Nathanson[1] supposed the plasma membrane to be a mosaic of lipoids and "protoplasm," but it is evident that if the lipoid portion is not continuous, it can not make the cell impermeable to any substance.

Czapek[2] states that lipoid solvents cause cytolysis when the surface tension of the solution is reduced to .68, and concludes from this that the plasma membrane contains glycerine tri-oleate since its emulsion reduces the surface tension of water to this figure.

The diffusion of water-soluble substances through swollen-plates, "gels" or "sols" of gelatine, varies inversely with the viscosity (Arrhenius). The great hysteresis of gelatine gels is taken advantage of to show that diffusion depends on the viscosity and not on the per cent. of gelatine, at the same temperature.[3]

The absorption of water by a gelatine plate increases its permeability, and the temperature and therefore the presence of substances which affect this swelling of gelatine affect its permeability. Impregnation of colloidal membranes with bile salts, alcohol, ether, acetone or sugar changes (usually increases) their permeability. The effects of substances on the rate of diffusion through gelatine plates, and on their swelling (viscosity) and melting point are not always quite parallel.[4]

In case the substance added to the membrane is removable, the change in permeability becomes reversible, which is true in regard to many of the substances mentioned above. Changes in non-living membranes are usually only partially reversible or are irreversible. Denaturalization of a colloid membrane by heat, heavy metals, or other coagulative agents which induce chemical changes in the membrane, or the addition of substances which cannot be removed, produce irreversible changes in permeability.

That the permeability of the membranes in living tissue is increased at death is proven by a host of observations. The electric conductivity increases enormously at death. Contained

[1] *Jahrb. wiss. Bot.*, 1903, XXXVIII., 284; 1904, XXXIV., 601, and XL., 403.

[2] *Ber. deutsch. bot. Gesell.*, 1910, 28, 480.

[3] Zangger, Asher & Spiro's *Ergeb. der Physiol.*, 1908, VII., 99.

[4] Zangger, *loc. cit.*

substances diffuse out, substances in the medium (fixing fluids, stains, etc.) diffuse in. There is a more general mixing of tissue substances. Enzymes come in contact with proteids and autolysis results.

Certain substances are known to increase the permeability of membranes in tissues of the body. Thus ether, chloroform, etc., increase the penetration of fixing fluids, and the exit of contained substances, and the mixing of tissue substances. In this way they increase autolysis.

II. Osmotic Phenomena in Plants.

It is evident that water, salts, carbon dioxide and oxygen can, at least occasionally, penetrate plant cells, as otherwise no growth could occur. In case of the higher plants, the same is true of sugars and other bodies.[1] Janse[2] found that so much KNO_3 is absorbed by *Spirogyra* cells in 10 minutes, that it may be easily detected microchemically with diphenylamin-sulphuric acid.

Osterhout[3] grew seeds of *Dianthus barbatus* in distilled water. The rate of growth during the several days of observation was normal. In nature, calcium oxalate crystals are found in the root hairs, but are not formed in the distilled water cultures, showing that the Ca comes from the medium. If placed in calcium solutions, crystals became large enough to see with the polarizing microscope in four hours, showing permeability to Ca.[4]

Nathanson[5] found that nitrates and other substances entered the cell. Ruhland also observed penetration of salts.

Traube-Mengarini and Scala[6] conclude that salts enter plant cells only through the partition walls. At these places there appears an "acid reaction" (bluing of methyl violet). They

[1] See Laurent in Livingstone, "The Rôle of Diffusion and Osmotic Pressure in Plants," 1903, p. 67.

[2] *Versl. en Medeel. der Konikl. Akad. van afdeel. Naturs.*, 3. Reeks, IV. part, 1888, p. 333.

[3] *Zeits. f. physik. Chem.*, 1909, LXX., 408.

[4] But compare von Mayenberg, *Jahrb. f. wiss. Bot.*, XXXVI., 381, who found little penetration of salts into fungous hyphæ. And see Demoussy, *Comptes Rendus*, CXXVII., 970.

[5] *Jahrb. wiss. Bot.*, XXXVIII., 284; XXXIX., 601; XL., 403.

[6] *Biochem. Zeit.*, 1909, XVII., 443.

interpret this as showing that the anion of the salt unites with an H ion of an amino group, forming a free acid, and the kation of the salt unites with the protoplasm. It appears to me that the basis of this conclusion is very slight.

Permeability may be investigated by a study of plasmolysis, which consists in the shrinkage of the surface protoplasm away from the cellulose cell wall, due to the osmotic pressure of the hypertonic solution of a dissolved substance which does not penetrate. A regaining of turgor by the cell while in the hypertonic solution indicates slow penetration of the substance. The plasmolytic method was originated by Nageli, who also noted that a shrinkage resembling plasmolysis but accompanied by outward diffusion of dissolved substances, occurs at death or severe injury to the cell.[1]

The plant cell is surrounded by an elastic cell wall. The internal osmotic pressure may be divided into three resultants: that causing rounding up of the cell is called turgor, that resulting in stretching of the cell wall is sometimes distinguished as turgescence, and that resisting the surface tension of the cell, "central pressure."

The plasmolytic experiments of DeVries[2] and others[3] are interpreted by them as indicating a selective impermeability of the plasma membrane to neutral salts.

In the plasmolytic experiments of Overton[4] all salts plasmolyzed permanently. Non-electrolytes fell in four groups, thus: Cane sugar, dextrose, manit, glycocoll > urea, glucerin > ethylene-alcohol, acetamid > methyl-alcohol, acetonitril, ethyl-alcohol, phenol, aniline, isobutyl-alcohol, isoamyl-alcohol, methyl acetate, ethyl acetate, butyl aldehyde, acetone, acetaldoxim. Diffusion of substances of homologous series increased with molecular weight.

Overton ascertained the permeability of plant cells to alkaloids

[1] "Pflanzenphysiol. Untersuchungen," 1885.

[2] *Zeit. physikal. Chem.*, 1888, II., 415; 1889, III., 103.

[3] Cf. Livingstone, "The Rôle of Diffusion and Osmotic Pressure in Plants," Chicago, 1903; Janse, *Bot. Centlb.*, 1887, XXXII., 21; Duggar, *Trans. Acad. Sc. St. Louis*, 1906, XVI., 473.

[4] *Vierteljahrschrift der Naturforschers. Gesell. in Zurich*, XLIV., 88; *Jahr. wiss. Bot.*, 1900, XXXIV., 669.

by their precipitation of the tannic acid in the cell sap. Most alkaloids penetrate rapidly, but only in the form of the free (undissociated) base produced by hydrolysis. Hence the penetration (precipitation and toxic effect) may be prevented by adding a little acid to the medium.

Pfeffer had shown that methylene blue is precipitated by tannic acid in the cell sap of certain plants.

Some discussion has arisen as to whether the mechanism of the entrance of dyes into plant cells is similar to that of alkaloids. Overton showed that lipoid soluble basic dyes penetrate easily. He at first supposed that only the free color base (undissociated) is able to penetrate the cell.[1] Overton found, however, that triphenylmethane and chinonimid dyes disprove his assumption, showing that it is at least not general. This question was taken up again by Harvey[2] who found that neutral red or methylene blue, which stain *Elodea* leaves in tap water, do not do so if just enough acid be added to the water to prevent any free color base from forming.

He observed that, although these dyes are not precipitated in the cell sap of this plant, they become more concentrated in the cell sap than in the medium. Neutral red is bright red in the cell sap, indicating that the reaction is acid (no free color base is present). He supposes that the absence of any of the dye in the form of the free color base prevents it from diffusing out of the cell, hence it becomes more concentrated within than without.

In using the plasmolytic method, if a cell does not recover from plasmolysis in a solution of a salt, it is said to be impermeable to that salt. However, the cell may recover, but may be killed by penetration of the salt, and shrink again. It is possible that Overton and others failed in some cases to note this transient recovery. Contrary to Overton, Osterhout[3] found *Spirogyra* permeable to alkali-salts and alkaline earth salts, but more

[1] In this connection it is interesting to note that Robertson observed that free color bases, and to a less extent free color acids, are much more soluble in fats than are their salts. This is what we should expect, since the salts dissociate in water, and ions are insoluble in fats.

[2] *Science*, 1910, n.s., XXXII., 565.

[3] *Science*, 1911, n. s., XXXIV., 187; XXXV., 112.

easily to Na than to Ca. It is plasmolyzed by .2*M* $CaCl_2$ and not by the isosmotic .29*M* NaCl but by .38*M* NaCl. .195*M* $CaCl_2$ and .375*M* NaCl just failed to plasmolyze. On mixing 100 c.c. .375*M* NaCl with 10 c.c. .195*M* $CaCl_2$, thus decreasing the osmotic pressure of the former, marked plasmolysis occurred. This indicates that Ca decreases the permeability to Na.[1] From further work by the same author, not yet published, it appears that Na increases and Ca decreases the permeability of certain marine plants. Also Fluri[2] obtained increase in permeability by salts of aluminium, yttrium and lanthanum.

DeVries plasmolyzed cells of *Tradescantia*, containing blue cell sap, with 4 per cent. KNO_3 solution, then added nitric acid until the color changed to red. The acid made the cells permeable to KNO_3 for they regained their turgor and finally burst. This explains the easy penetration of acids into cells. Pfeffer[3] found that if red beet cells, petals of *Pulmonaria*, stamen hairs of *Tradescantia* and other anthocyan-containing cells are placed in extremely dilute HCl or H_2SO_4, they suddenly turn red, indicating immediate penetration of the acid. If allowed to remain but a short time, the cells are not killed, and the color change is reversed on returning the tissues to acid-free water.

I have repeated these experiments, using cells of red beet, red cabbage and red nectar glands of *Vicia faba*, and find that mineral acids penetrate, but that (the lipoid soluble) acetic acid penetrates much more rapidly and also more easily alters the plasma membrane, causing pigment to diffuse out, if not cautiously applied. Alkalis also penetrate, but (the lipoid soluble) ammonia penetrates much more rapidly than the others. Ammonia does not so easily increase the permeability to the pigment as does acetic acid.

Ruhland[4] after staining root hairs of *Trianea*, etc., with the indicators, methyl orange and neutral red, found that mineral acids as well as lipoid soluble acids penetrated.

[1] The work of Kearney, Report 71, U. S. Dept. of Agriculture, indicates that Ca prevents the plasmolytic and toxic effect of Mg, but this is "false plasmolysis" following death.

[2] *Flora*, 1908, XCIX., 81.

[3] "Osmotische Untersuchungen," Leipzig, 1877, p. 135.

[4] *Jahrb. wiss. Bot.*, 1908, XLVI., 1.

One defect in the plasmolytic method is the fact that the cellulose cell wall, if not very thick, is elastic, and a slightly hypertonic solution may cause the cell to decrease in volume without pressing the protoplasm away from the cell wall. This source of error may be eliminated by substituting calculations of the volume of the cells (as necessary for animal cells) for observations on plasmolysis.

It is well known that movement, and in many cases increase in size of plants is due to changes in turgor of the cells. If we exclude the turgor changes in aerial plants produced by variations in the ratio of the water supply to the transpiration, turgor changes may be due to changes in the osmotic pressure of the external medium, or of the cell sap (due to metabolic changes) or to changes in the permeability of the plasma membrane. Lepeschkin[1] has confirmed Pfeffer in showing that changes in permeability of stipule cells accompany (or immediately precede) changes in turgor. By chemical analysis of the medium he has shown that an outward diffusion of dissolved substances, from the cells, accompanies loss of turgor, and by plasmolytic experiments, that the permeability to certain substances increases.

It is interesting to note the force that may be exerted by such changes in turgor. From measurements of the pull of a stamen hair of *Cynara scolumus* or *Centaurea jacea* on loss of turgor following stimulation, it seems not improbable that the change in turgor amounts to 2–4 atmospheres (Höber). This also indicates the strength of the cell wall necessary to prevent rupture of the plasma membrane. The osmotic pressure of the juices pressed out of plants varies from 3.5–9 atmospheres.[2] The pressing out of the juices causes an error due to chemical changes; on the other hand, in taking the freezing point or pieces of plant tissues, an error arises from lowering of the freezing point by the walls of the capillary spaces. Müller-Thurgau[3] found the Δ (corrected freezing point lowering) of plant tissues $= .8–3.1°$. Many plants respond to light by definite movements, produced

[1] *Ber. deutsch. bot. Gesell.*, XXVI. (*a*), 725.

[2] DeVries, *Pringsheime Jahrbucher wiss. Bot.*, 1884, XIV., 427; Pantanelli, *ibid.*, 1904, XL., 303.

[3] *Landwirtschaftl. Jahrb.*, 1886, XV., 490.

by turgor changes in certain of their cells. Trondle[1] found that light produced changes in permeability of these cells.

Changes in permeability may not only affect the turgor, but also the assimilation and excretion, and consequently the metabolism and growth of the cells. Chapin[2] observed that CO_2 in certain doses is a stimulant to the growth, not only of green plants but also of moulds. As only a few saprophytes can decompose CO_2, it is not probable that its effect is nutritive. A similar stimulating action of ether and various salts, even such toxic ones as those of zinc, was previously known. These salts probably stimulate without penetrating the cells, since Zn, for instance, is not a constituent of protoplasm.[3] This leads one to suppose that the initial effect of all of these substances is on the surface, changing the permeability of the cells.

Wächter[4] found that potassium decreases the permeability of onion cells. Sugar diffused out of sections of *Allium cepa* placed in distilled water or hypotonic sugar solutions, but a trace of potassium salt entirely prohibited the diffusion. When the K was removed the diffusion recommenced.

Czapek[5] determined increase in permeability by the exosmosis of tannin in cells of *Echeveria* leaves. Various monovalent alcohols and ketones, ether, ethyl urethan, di and tri acetin, Na-oleate, oleic acid, lecithin and cholesterin all just caused exosmosis of tannin in concentrations (aqueous solutions) which had a surface tension of about 0.68. It would appear therefore that these substances, chiefly of the class of indifferent narcotics, alter the cells if they diffuse into them, or diffuse into certain structures such as the cell lipoids or the plasma membrane. It seems more reasonable to suppose that the plasma membrane is the structure affected, and the more the substance lowers the surface tension of water, the more it diffuses into the plasma membrane. When this membrane is altered, it allows escape of tannin. Some substances such as chloral hydrate are effective

[1] *Jahrb. f. wiss. Bot.*, 1910, XLVIII., 171.

[2] *Flora*, 1902, XC., 348.

[3] Cf. Loeb, "Dynamics of Living Matter," pp. 73, 74.

[4] *Jahrb. wiss. Bot.*, 1905, XLI., 165.

[5] "Über eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzenzellen," Jena, G. Fischer, 1911.

in less concentration, and probably affect the cell chemically as well as physically.

Mineral acids caused exosmosis of tannin when the concentration just exceeded 1/6,400 normal, and the effect is probably due to H ions. At this same concentration Kahlenberg and True[1] found the growth of seedlings of *Lupinus albus* to cease. It appears, therefore, that this cessation of growth is due to increased permeability, causing decreased turgor of the cells.

Changes in permeability may also affect secretion (excretion). The addition or formation of alcohol or acetates causes yeast and other fungi to secrete (excrete) for a short time, various substances, especially enzymes which do not come out in a culture medium lacking the reagent.[2] It appears that the alcohol or acetates increase the permeability of the fungi to these substances.

My own experiments[3] indicate that pure $MgCl_2$ solutions increase the permeability of yeast. A certain per cent. of yeast and dextrose in .3 molecular $MgCl_2$ eliminated CO_2 more rapidly than .5*M* NaCl or .325*M* $CaCl_2$, all which have about the same freezing points. Also, the CO_2 elimination was more rapid in the magnesium solution than in a solution of the same concentration of $MgCl_2$ with either of the other salts in addition, or in a solution containing NaCl and $CaCl_2$ in the same concentrations as in their respective pure solutions, or in a solution of all three salts, or in tap or distilled water. In order to determine whether the magnesium entered the cells I took two equal masses of compressed yeast and agitated one in H_2O and the other in a molecular solution of $MgCl_2$ for 5 hours, then washed each rapidly in H_2O by means of the centrifuge. The ash of the magnesium culture = .048 gram, that of the control = .0466 gram. Evidently the Mg did not enter the yeast to any great extent, and probably acted on the surface, increasing the permeability.

Ewart[4] observed that after placing plant tissue in 2 per cent. HCl and washing in water its electric conductivity (ionic permeability) was increased. If one portion of the plant is stimulated, the stimulus may be transmitted to other portions. In

[1] Kahlenberg and True, *Botanical Gazette*, 1896, XXII., p. 81.

[2] Zangger, "Asher and Spiro's Ergeb. d. Physiol.," 1908, VII., 144.

[3] McClendon, *Am. Jour. Physiol.*, 1910, XXVII., p. 265.

[4] " Protoplasmic Streaming in Plants," Oxford, 1903, p. 96.

this way increase in electric conductivity was produced by stimulation of a point outside the path of the current.

Whereas many plants are very sensitive to sudden and extreme changes in osmotic pressure, Osterhout[1] found that certain marine algæ thrived when subjected daily to a change from fresh water, to sea water evaporated down until it crystallized out, and vice versa. He does not state whether these algæ survive extreme plasmolysis, or whether they are so easily permeable to salts as not to be plasmolyzed by the saturated sea water or burst by the fresh water.

For regulation to slight changes in the osmotic pressure of the medium, a change in size of the cell altering the turgescence, or tension of the cell wall, is sufficient.

If *Tradescantia* cells are placed in a hypotonic solution, they begin to swell. But soon crystals of calcium oxalate are formed in the cell sap, and in this way the turgor, due chiefly to oxalic acid, is reduced.[2] It would be interesting to know what is the source of the Ca. Was it previously in combination with proteids?

The accommodation to a hypertonic medium takes place, according to van Rysselberghe, partly through absorption of substances of the medium and partly through metabolic production of osmotic substances, chiefly the transformation of starch into oxalic acid.[3]

III. Bio-electrical Phenomena.

1. *In Plants.*

Change in permeability of the plasma membrane to ions would necessarily cause electrical change due to its influence on the migration of ions. These electrical changes actually occur, and may be easily studied.

Stimulation or wounding in plants is accompanied by an electronegative variation of the affected surface. This negative region spreads in all directions over the surface, but the rate of

[1] Univ. of Cal. Pub., Bot., 1906, II., 227.

[2] Van Rysselberghe, Mém. d. l'Acad. royale de Belgique, 1899, LVIII., 1.

[3] Compare von Mayenberg *Jahrb. f. wiss. Bot.*, XXXVI., 381.

propagation[1] is much slower than the similar process in muscle or nerve.[2]

Pfeffer[3] supposed that the plasma membrane is normally permeable to ions of only one sign. Since the normal cell surface is positive in relation to the cell interior (cut surface) we may conclude that the plasma membrane is normally more permeable to kations (less permeable to anions). Just as the negative variation of wounding is due to the removal or rupture of the plasma membrane, so the negative variation of stimulation would, on the membrane hypothesis, be due to increase in permeability of the plasma membrane to the confined anions.

An alternative hypothesis is that these electrical changes result from changes in metabolic activity. The production of an electrolyte whose anion and kation have very different speeds of migration (such as an acid or alkali) would cause electrical changes. But how are we to account for changes in metabolic activity? There exists varied evidence for changes in permeability, and it is simpler to assume that changes in metabolic activity and electrical changes are both the result of changes in permeability.

Kunkel[4] tried to explain the vital electrical phenomena as the result of the movement of fluids in the vessels of the tissues, but bio-electrical changes may occur without such movement of fluids (Burdon-Sanderson).

Kunkel observed in 1882[5] that the movement of the leaf of *Mimosa pudica* is accompanied by an "action current," or negative variation of one surface of the pulvinus. Similar results on *Dionœa* leaves were obtained by Munk[6] and specially studied by Burdon-Sanderson.[7] It was stated above that Lepeschkin had shown that the turgor changes in plants were accompanied or immediately preceded by changes in permeability to certain substances. The electrical phenomena suggest that the turgor

[1] Which is in *mimosa* 600–1,000 times as fast as the geotropic impulse in a root.

[2] Fitting, "Asher and Spiro's Ergeb. d. Physiol.," 1906, V., 155.

[3] "Pflanzenphysiologie."

[4] *Arch. f. d. ges. Physiol.*, 1881, XXV., 342.

[5] See Winterstein's "Handbuch der vergleichenden Physiologie," III. (2), 2, p. 214.

[6] *Arch. f. Anat. u. Physiol.*, 1876, XXX., 167.

[7] *Proc. Roy. Soc. London*, 1877, XXV., 441; *Philos. Trans.*, 1888, CLXXIX., 417.

change is accompanied (or immediately preceded) by increase in permeability of the plasma membrane to anions. Burdon-Sanderson states that, whereas the movement resulting from turgor change begins 2.5 seconds after stimulation, the negative variation reaches its maximum 1 second after stimulation. This may be due to the mechanical inertia, or the time required for the diffusion of substances.

It was stated in the preceding chapter that light changes the permeability of the plasma membrane, and Waller[1] found corresponding electrical changes due to light, but not always in the same direction in different plants. This inconstancy in direction is probably due to the fact that light not only influences the permeability, but also the assimilation, and changes in assimilation produce electric changes. This is supported by the fact that Querton[2] found that assimilation as well as electric change is most affected by the longer light rays.

2. *In Muscle and Nerve.*[3]

Ostwald[4] proposed the hypothesis that the electric phenomena of muscle, nerve and the electric organs of fish (which may reach several hundred volts) are produced with the aid of semipermeable membranes. The alternative theory of Hermann, which would account for the current of injury by assuming the production of some electrolyte (alkali?) in the wounded region, whose anions and kations have very different speeds, seems less probably to be the correct one.

According to the "membrane theory," the muscle or nerve element is surrounded by a semipermeable membrane allowing easier passage to kations than to anions. The kations passing through the membrane are held back by the negative field produced by the confined anions, but owing to their kinetic energy, the kations pass out far enough to give the outside of the cell surface a positive charge. Therefore any portion of the surface that is made freely permeable to anions becomes electronegative

[1] *Jour. of Physiol.*, 1899–'00, XXV., 18.

[2] "Contribution à l'étude du mode de la production de l'électricité dans etres vivantes," *Travaux de l'Institut Solvay*, 1902, V.

[3] Cf. R. Lillie, *Amer. Jour. Physiol.*, 1911, XXVIII., 197.

[4] *Zeit. physik. Chem.*, 1890, VI., 71.

in relation to the remainder of the surface. This negative variation may be produced by artificially removing or altering a portion of the membrane (producing the current of injury) or as the result of normal stimulation, making it permeable to anions (action current).

Bernstein resorted to mathematical proof of this hypothesis. We will not here go into details, but the gist of the matter is that if the process were as we have imagined it, the electromotive force of the current of injury, or action current, should be proportional to the absolute temperature. He found this to be true for temperatures between 0° and 18°, but between 18° and 32° the E.M.F. was found to be too small. The muscle was not permanently injured by exposure to the higher temperatures for the length of time necessary for the experiments. Bernstein explained this discrepancy by the further assumption that at the higher temperatures the plasma membrane became slightly more permeable to anions.[1]

Since the muscle contains a higher per cent. of potassium than the blood plasma or lymph, it might be supposed that K ions passed outward through the plasma membrane and gave the surface of the muscle element the positive charge. But if this were the case, the current of injury should be reversed by placing the muscle in a solution containing potassium in greater concentration than in the muscle. This reversal, however, was shown by Höber not to occur. Since lactic and carbonic acids are produced by muscle and diffuse out in increased amount on contraction, one might suppose H ions to give the muscle surface the positive charge. This is only a guess (and a poor one, since undissociated molecules of CO_2 and lactic acid are lipoid-soluble) but may be convenient until some better one is proposed. Perhaps the carbonic acid combines with amphoteric proteids, which

[1] This is similar to the conclusion reached by Biataszewicz, *Bull. d. l'Acad. d' Sc. d. Cracovie, Sc. Math. e. Nat.*, Oct., 1908, p. 783, in regard to the unfertilized frog's egg. In order to explain his observation that the rate of swelling in tap water increased 5 times for every 10° rise in temperature, he assumed that heat increased the permeability to H_2O. This would seem to be the simplest explanation, provided the swelling were not due to chemical production of osmotic substances: and since the Δ of the ripe ovarian egg is .48° but is reduced to .045° after oviposition, *Biochem. Zeit.*, 1909, XXII., 390, much if not all of the swelling is probably due to the initial osmotic pressure of the egg interior.

then set free H^+ and HCO_3^- ions, thus increasing the ionization and therefore reducing the number of undissociated molecules, which can escape.[1]

Since Osterhout showed that certain electrolytes may alter the permeability of cells, we might expect to find, on the membrane hypothesis, an effect of salts on the electric polarization of muscle. Höber[2] observed that a portion of the surface of a muscle treated with certain salts, KCl for instance, becomes electro-negative (more permeable to anions) whereas a portion treated with NaI or LiCl becomes positive (still less permeable to anions than is the normal unstimulated muscle). The order of effectiveness of the ions is as follows: $Li < Na < Cs < NH_4 < Rb < K$ and $CNS < NO_3 < I < Br < Cl <$ valerianate, butyrate, propionate, acetate, formate $< SO_4$, tartrate. Similar ionic series were found by Overton, R. Lillie, Schwartz, Mathews, Grützner, Höber, and Mayer in the effect of salts on the functional activity of muscle, nerve and cilia, but the exact relation of these phenomena to permeability is not understood in every case. Pure solutions of salts of alkali metals may "inhibit" muscle by *increasing* permeability, but salts of alkali earth metals are said to "inhibit" by *decreasing* permeability.. Mayer says that the effect of salts on cilia is the reverse of that of muscle, but the relation of this to permeability is not known. Since ions affect the aggregation state of hydrophile colloids in the same or exactly reversed order, and the kation series is found in no other known physico-chemical phenomena, it might be supposed that the semipermeable membranes of muscle are colloidal.

It seems probable that sugar solutions inhibit the activity of muscle by increasing the permeability, but since sugar is not an electrolyte this question cannot be tested by electric methods.

A negative variation of muscle may also be produced by the so-called "hæmolytic" substances, but is irreversible, whereas that produced by salts may be reversible. In this connection it

[1] Roaf, *Q. J. Exper. Physiol.*, 1910, III., 171, supposed the anion to be protein; however it has not been shown that proteids, or even amino acids diffuse out on stimulation. I do not see that the speculation of Galeotti, *Zeit. f. Allgem. Physiol.*, 1907, VI., 99, is at all explanatory.

[2] *Loc. cit.* and *Pflüger's Arch.*, 1910, CXXXIV., 311.

is interesting to note that Overton[1] found the permeability of muscle to be similar to that of plant cells.

It might appear to the reader that the membrane theory is merely wild speculation. What proof have we that on injury or during contraction the muscle is more permeable to any ion?

DuBois Reymond[2] and Hermann[3] explained the fact that living muscle has a greater electric resistance than dead muscle on the hypothesis that the resistance of living muscle is due to the presence of membranes, which become more permeable at death. They demonstrated the resistance of muscle tissue to the passage of ions by the fact that electric polarization occurs in muscle tissue on the pasage of an electric current. It seems to me that Kodis[4] and Galeotti[5] take a step backward, in attributing the decreased resistance of dead muscle to the liberation of ions. Galeotti tried to support his view by determinations of the freezing points of the living and dead muscle, but found on the contrary that the change in electric conductivity of the muscle did not correspond to the change in the osmotic pressure.

Du Bois Reymond[6] observed that the electric conductivity of muscle changes on (during?) contraction and Galeotti[7] found it to be greater on strong contraction than on weak contraction, and least on fatigue-exhaustion or cold-anæsthesia. However, the duration of a contraction is momentary (about 1/5 second for frog's muscle) and it is not clear that these investigators measured the conductivity accurately during such a brief period, in fact they probably measured it after contraction. Therefore I decided to repeat these experiments, using a method by which I could measure the conductivity during the actual contraction period, as well as in the unstimulated condition.[8]

[1] *Pflüger's Arch.*, 1902, XCII., 115.

[2] "Untersuchungen über thierische Electricität," 1849.

[3] *Pflüger's Arch.*, 1872, V., 223, VI., 313.

[4] *Am. Jour. Physiol.*, 1901, V., 267.

[5] *Zeit. f. Biol.*, n. f., 1902, XXV., 289; 1903, XXVII., 65.

[6] *Loc. cit.*

[7] *Loc. cit.*

[8] McClendon, *American Journal of Physiology*, 1912, XXIX., 302.

Experimental.

Platinum electrodes, platinized with platinic chloride containing a little lead acetate, and of a form similar to those designed by Galeotti, were used. Galeotti stimulated the muscle through the same electrodes used in measuring the electric conductivity, by switching on a different electric current. Though it were possible to throw a switch quickly enough to have the current for measurement of conductivity pass through the muscle during contraction, it would be necessary to use a string galvanometer to take the reading, and this method would probably not be very accurate. A more accurate method is that of Kohlrausch, in which a rapidly alternating current reduces polarization at the electrodes and in the tissue, but it is necessary to throw the muscle into tetanus in order to have time for the reading. I accomplished this by using the same current for stimulation and measurement of conductivity. A very small induction coil was fitted with a rheostat in the primary. Another rheostat in the secondary could be thrown out of the circuit by a switch. By adjusting the rheostats, a current strong enough to be distinctly heard in the telephone, yet too weak to stimulate the muscle, was obtained. By switching the resistance out of the secondary circuit, the current could immediately be increased so as to throw the muscle into tetanus. Since the Wheatstone bridge was used, the difference in current strengths had no direct effect on the readings. The conductivity increased from 6 to 28 per cent. (being usually about 15 per cent.) on stimulation.

We have, then, evidence for the increase in permeability of muscle to ions during contraction, but what relation has this to the mechanism of the contractile process? It has been suggested by D'Arsonval, Quincke, Imbert, Bernstein, Galeotti and others that the increased permeability to ions causes a disappearance of the normal electrical polarization of the elements, whose surface tension consequently increases, causing them to round up (shorten). But what are the elements concerned? It would be confusing to assume them to be the fibers, as then the function of the complicated internal structure would be unexplained. They are probably not the sarcous elements (portions of fiber between 2 Z-lines) as the rounding up of these ele-

ments would elongate the muscle. And even though contraction were produced by inequality in surface tension, as assumed by Macallum[1] the total surface change would be so small as not to account for the energy liberated in contraction. In order to avoid this last difficulty Bernstein made use of hypothetical ellipsoids. These were surrounded by elastic material to account for elongation of the muscle.[2]

The great differences of potential (several hundred volts) that may be produced by the electric organs of fish, is achieved by the arrangement of the modified muscle plates in series. All of the plates have the nerve termination on the same side. On stimulation of the nerve, each plate becomes negative, first on the nerve termination side, and thus the negative side of one plate touches the positive side of the next plate. In this way the direction of the current may be determined by studying the anatomy of the innervation. This rule, discovered by Pacini, finds an exception only in *Malopterurus*, whose electric organ is supposed by Fritsch to be derived, not from muscle but from skin glands.

The electric fish are *relatively* immune to electric currents passed through the medium. This is not merely an apparent immunity due to the fish being out of the path of the current, or the current being short circuited by sea water (in case of marine fish). I have received severe shocks from a torpedo that was entirely submerged in sea water.

3. *Amœboid Movement.*[3]

The normal unstimulated surface of plant and animal tissues is electro-positive in relation to the cut or injured surface of the cells. We have given reasons for assuming that this indicates greater permeability of the plasma membrane to kations than to anions, the latter accumulating in the cell interior, gives it a negative charge.

There are two reasons for believing that this is true also of the *Amœba:*

[1] *Science*, n. s., 1910, XXXII., 822.

[2] Meigs., *Am. Jour. Physiol.*, 1910, XXVI., 191, supposes the rounding up of muscle elements due to increased turgor.

[3] McClendon, *Arch. f. d. ges. Physiol.*, 1911, CXL., 271.

1. If a weak electric current is passed through water in which an *Amœba* is suspended, it is carried passively toward the anode, indicating that it has a negative charge. This charge may be due to confined anions.

2. If a stronger electric current is passed through an *Amœba*, it begins to disintegrate first at that surface nearest the anode. The disintegration is probably due to the accumulation of ions retarded by the plasma membrane. The ions in the medium are free to pass around the *Amœba*, but the contained ions must pass the plasma membrane in order to migrate to the electrodes. Since the disintegration is toward the anode, it is probably due to anions which cannot get out of the *Amœba*. Since no corresponding disintegration begins toward the kathode, the plasma membrane is probably more permeable to kations.

The surface tension of the *Amœba* is very low, and apparently increases on strong stimulation (indicated by rounding up of the *Amœba*). We saw that stimulation in plant and muscle cells caused increased permeability to ions, and consequently disappearance of the normal electrical polarization, and thereby causing increased surface tension. We might conclude therefore that the low surface tension of the *Amœba* is caused by electric polarization, due to the production of some metabolic electrolyte whose anions cannot escape; and that strong stimulation causes increased permeability and hence disappearance of the electrical polarization.

This would explain all negative tropisms of the *Amœba*. The surface tension of the portion most strongly stimulated is increased, and the *Amœba* flows away from the stimulus.

In order to explain positive tropisms we would have to make another assumption. If the stimulus did not act directly on the plasma membrane, but penetrated the *Amœba* and acted on the protoplasm, and increased the production of the metabolic product producing polarization of the plasma membrane, it would thereby decrease the surface tension. The local decrease in surface tension would cause the *Amœba* to flow toward the source of the stimulus, just as the quicksilver drop in dilute HNO_3 flows toward potassium bichromate in Bernstein's experiment.

All stimuli producing positive tropism would then have to penetrate to a greater or less distance into the *Amœba*. But the same stimulus thus acting on the interior might, in greater intensity, affect also the plasma membrane, increasing its permeability and changing the positive to negative tropism. Such a change of the sign of tropism has been observed.

Soap lowers the surface tension of fats and lipoids, and Quincke, Bütschli, Loeb, Robertson and others supposed that lowering of the surface tension of living cells might be due to soap. However, I found that soap always causes negative tropism in *Amœba*, probably because it increases the permeability of the plasma membrane.

4. *The Propagation of the Bio-electric Changes.*

On the hypothesis, that the electric phenomena in muscle and nerve, as well as other animal and also plant tissues, is due to change in permeability to ions, we might hope to explain the wave-like propagation of these changes. Since extraneous electric currents "stimulate" all tissues (presumably by increasing permeability) thus causing them to produce additional electric phenomena, it seems natural that these latter would be self-propagating. It is probably the negative variation of nerve which stimulates the muscle, and the negative variation of the portion of the muscle fiber adjoining the nerve ending, which stimulates the adjacent portions of the muscle. Nernst found mathematical proof that electric stimulation is due to change in ionic concentration at the semipermeable membranes.

I have found evidence that the negative variation (current of injury) in plants, may strongly affect adjacent cells. If an electric current of suitable density is passed through plant or animal tissue, negatively charged colloids in the protoplasm migrate toward the anode. I have observed this movement in living cells, and the resulting displaced bodies in histological sections. In certain cases there may be some doubt whether the colloids moved toward the anode, or water toward the kathode, but in others, easily distinguishable bodies such as chromatin granules or threads moved toward the anode.

If the tip of a root be cut off we observe a negative variation

of the cut surface. This produces an electric current through the medium and surrounding tissue. The fact that the current actually passes through adjacent cells is shown by a displacement of their contained colloids, identical in appearance with the displacement produced by the currents used in the above experiments. Nêmec[1] apparently observed these changes but did not correctly describe or interpret them.

The fact that an electric current on increase (make) stimulates muscle at the kathode, and the fact that the muscle surface is normally positive in relation to the interior (cut surface), probably indicates that stimulation is produced by a rapid depolarization of the muscle surface.

If this reasoning be applied to an individual contractile element, we may assume that the current causes kations to leave the outer surface of the membrane, and other kations to be attracted toward the inner side of the membrane, and thus the polarization disappears or may even be reversed. Just how this causes an increase in permeability of the membrane is a matter which we will leave to the future for discussion.

It has been supposed that the stimulated region acts as kathode to the adjacent portions, and these in turn act as kathodes to the next portions and so the stimulus is propagated.

Stimulation of a part of the surface, causing it to become more permeable to ions, depolarizes the adjacent parts of the surface owing to the fact that confined anions migrate through the permeable region and neutralize the charges of the kations on adjacent parts of the impermeable region (see Fig. 1). For this reason the increase in permeability is propagated.

This explanation of the phenomenon in a single element holds for a tissue made up of many elements provided these are in contact, as illustrated by the accompanying Fig. 2. This is probably the mechanism of propagation of the negative variation (and "stimulus") in many plant and animal tissues.

This mechanism accounts for the movement of the negative variation over a surface. But it may be possible for this electric change to jump from one element, to another not touching it. The observations on the current of injury, cited above, show that

[1] "Reizleitung u .d. reizleitenden Strukturen b. d. Pflanzen," Jena, 1901.

increased permeability of part of a tissue surface, may cause electric currents to flow through cells some distance from the wound. These currents probably stimulate the cells through which they pass, which in turn become permeable and produce electric currents. This explains the propagation of stimuli

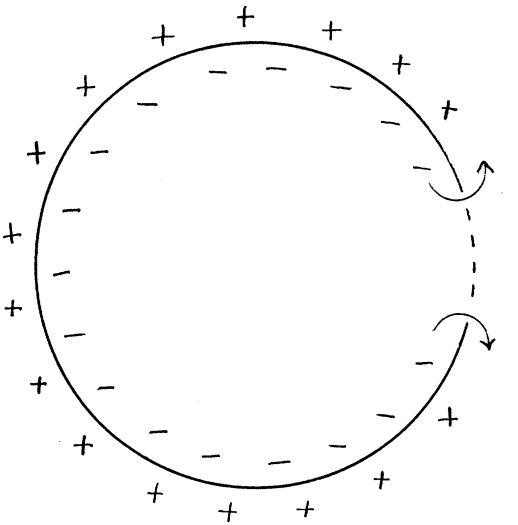

FIG. 1.

Anions represented by minus sign, kations represented by plus sign. Arrows denote the direction of migration of ions. The large circle represents the plasma membrane, the dotted line denoting the permeable and the continuous line, the impermeable portion.

through loose tissues, and the structural changes, as observed by Nêmec.

The rate of propagation of the "wound stimulus" is very slow, whereas that of propagation of the "stimulus" (negative variation) in sensitive plants is more rapid, and that of the nerve impulse still more rapid. We have not, however, sufficient data to show whether this is a mathematical objection to the hypothesis.

The streaming movements in plants may be stopped by a strong stimulus or "shock." This stimulus is usually propagated in one or more directions. Ewart[1] states that the rate of propagation at 18° in a single elongated cell of *Nitella* is 1–20 mm.

[1] *Loc. cit.*

per sec., but where it has to pass cell walls .001–.03 mm. per sec. However, the stoppage of the streaming was his criterion of the presence of the stimulus, and probably the banking of the stream

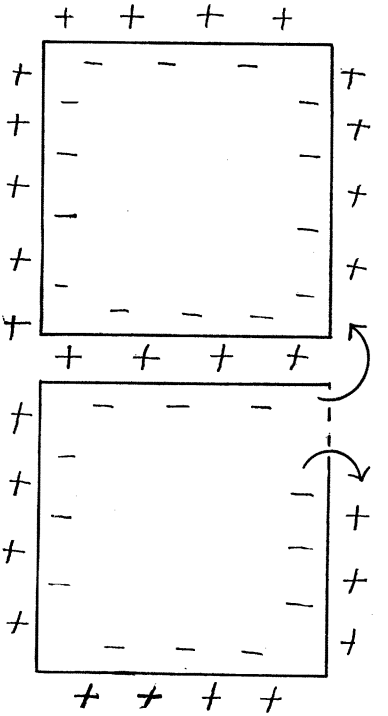

FIG. 2.

The squares represent the plasma membranes of adjacent cells. For further explanation see Fig. 1.

at one point, soon stopped the whole stream thus simulating the propagation of the stimulus.

IV. NARCOSIS.

If stimulation consists in increase in permeability, we should expect anæsthetics to prevent this change. The object of this chapter is to present evidence that may support or refute such a hypothesis.

Overton observed that warm- and cold-blooded vertebrates, insects and entomostraca, require practically the same concentration of the anæsthetic for narcosis. Certain groups of

worms require double, and protozoa and plants six times this concentration. We might conclude from this that nerves (and especially medullated nerves?) are more susceptible to narcosis than are other cells. All groups of worms contain nerves, but Loeb has shown that certain worms may perform coördinated movements after the nerves are cut, hence the higher concentration of the narcotic required to quiet them. However it should be remembered that *over-stimulation* causes rounding up and quiescence of *Amœba* and muscle may be paralyzed by increasing the permeability. The growth of plants is increased by a certain concentration of ether and retarded by a greater concentration. It may be that true narcosis (decreased permeability) of protozoa and plants cannot be produced by such substances as ether, etc.

Vertebrate nerve tissues are rich in lipoids (which have similar solubilities to neutral fats) and it is therefore significant that Overton and also Meyer[1] found that the partition coefficient of anæsthetic between olive oil and water corresponds to its anæsthetic power. Meyer[2] showed further, that with change of temperature, the change in the partition coefficient between oil and water, and the anæsthetic power of the substance were parallel. Pohl, Frantz, Gréhaut, and Archangelsky found that chloroform, ether, alcohol, chloral-hydrate or acetone, became more concentrated in the central nervous system than in other tissues. This is probably due to the absorption of the narcotic by the lipoids (especially the immense mass of myelin) in the nerve tissues.

If it could be proven that the plasma membrane consists of lipoids, this solubility of narcotics might be considered direct evidence for or against the permeability hypothesis, but lacking such proof we must first attack the subject from another side.

Höber[3] observed that ethyl-methane, phenyl-methane, chloral-hydrate, chloroform and hypnon, in *low concentration* prevent the production by salts, of the current of injury on muscle. He showed that in *lethal doses* on the contrary these narcotics do

[1] *Arch. exp. Path. u. Pharm.*, 1889, XLII., 109.

[2] *Arch. exp. Path. u. Pharm.*, 1901, XLVI., 338.

[3] *Pflüger's Arch.*, 1907, CXX., 492, 501, 508. Cf. R. Lillie, *Am. Jour. Physiol.*, 1912, XXIX., 373.

not prevent but even *produce* a current of injury, in this way explaining data which might otherwise seem to contradict the first statement. Galeotti and Cristina[1] observed that ether, ethyl-chlorid, and chloroform produce a current of injury on frog's muscle.

We may conclude, then, that anæsthetics, in the concentration producing narcosis, so change the plasma membrane as to prevent salts from making it permeable to anions. This is probably also true of nerve, since Höber found that ethyl-methane in low concentration prevented the sensitizing of nerve with K_2SO_4.

Höber has attempted to connect these facts with the lipoid solubility of narcotics. Moore and Roaf[2] had observed that *small quantities* of such narcotics as chloroform, alcohol, ether, or benzol, precipitated lipoids extracted from organs and suspended in water. But Höber and Gordon[3] found that colloidal solutions of lecithin were not precipitated, but were made transparent by ether or chloroform in *high concentration*. Similarly, Goldschmidt and Pribram[4] observed that lecithin suspended in NaCl solution, which is dissolved by chloral hydrate, methane, or cocaine, in high concentration, is precipitated by them in low concentration. On the other hand, Koch and McLean[5] state that chloral, hypnon, acetone, or pure ether, do not change the size of colloidal particles of lecithin (*i. e.*, make them easier or more difficult to salt out). Calugareanu[6] explains the mechanism of the precipitation of lipoids by anæsthetics by the increase in size of the particles due to absorption of the anæsthetic.

Thus there seems to be a parallel difference between the action of low and high concentrations of anæsthetics on muscle and nerve, and the action of the same on lipoid suspensions, but this does not hold true for all cases. Moore and Roaf[7] conclude that anæsthetics are bound, not only by lipoids, but also by proteids,

[1] *Arch. allg. Physiol.*, 1910, X., 1.

[2] *Proc. Roy. Soc. London*, 1904, LXXIII., 382; 1906, LXXVII., 86.

[3] *Hofmeisters Beitrage*, 1904, V., 432.

[4] *Zeit. f. exper. Path. u. Ther.*, 1909, VI., 1.

[5] *Jour. Pharm. and Exp. Ther.*, 1910, II., 249.

[6] *Biochem. Zeit.*, 1910, XXIX., 96.

[7] *Loc. cit.*

and their charactersitic action on the permeability of the living cell may be due to their action on proteids. In other words, the plasma membrane may be entirely proteid.

It is well known that during narcosis little or no oxygen is absorbed by nerve tissue. Verworn and his pupils assumed that the narcotic directly suppressed oxidation. On the other hand Mansfeld[1] supposed that the narcotic dissolving in a lipoid plasma membrane made it less permeable to oxygen. It would be more in harmony with the phenomena considered in previous chapters, to suppose that the narcotic in low concentration decreased the permeability of the plasma membrane to the anions and molecules of some acid end product of oxidation, and thus stopped the combustion. An objection to this hypothesis is made by Warburg[2] who found that phenylurethan, which only slightly reduces oxidation in certain cells, fertilized eggs, delayed cell division enormously. With greater concentration of the narcotic, oxidation was greatly reduced.

V. Osmotic Properties of the Blood Corpuscles.

Hamburger and Bubonavik[3] have concluded that the erythrocytes are permeable to K, Na, Ca and Mg. However, the opposite conclusion was reached by previous workers.

Gyrn's,[4] Hedin,[5] Traube[6] and others observed that the erythrocytes are relatively impermeable to neutral salts (exc. NH_4 salts) amino acids, various sugars and hexite, slowly permeable to erythrite, more permeable to glycerine, and easily permeable to monovalent alcohols, aldehydes, ketones, esters, ether, and urea. In general, it may be said that the erythrocyte is permeable to lipoid-soluble substances or those that lower the surface tension of water. Such substances (for instance, ether) become more concentrated in the corpuscle than in the serum. Saponin becomes 120, and ammonia 880 times more concentrated in corpuscle than in serum.[7]

[1] *Pflüger's Arch.*, 1909, CXXIX., 69.

[2] *Zeit. physiol. Chem.*, LXVI., 305.

[3] *Arch. internat. de Physiol.*, 1910, X., 1.

[4] *Pflüger's Arch.*, 1896, LXIII., 86, and *Koninkl. Akad. von Wetensch. Amsterdam*, 1910, p. 347.

[5] *Pflüger's Arch.*, 1897, LXVIII., 229; 1898, LXX., 525.

[6] *Biochem. Zeit.*, 1908, X., 371.

[7] Arrhenius, *Biochem. Zeit.*, 1908, XI., 161.

The erythrocytes are practically impermeable to ions. Stewart[1] observed that they offered a great resistance to the electric current. It is difficult to remove all of the serum from a mass of erythrocytes, but Bugarsky and Tangl, working independently of Stewart, obtained sediments of corpuscles having a conductivity of only 1/50 that of the serum. This indicates that the corpuscles are practically impermeable to both classes of ions, for if permeable to ions of one sign, they would probably not be such good insulators. The electric conductivity of the ash (made up to equal volume) of the corpuscles is about that of the serum, although the osmotic pressure of the solution of ash of the latter is greater.[2]

Hence an increase in electric conductivity of the corpuscles (as will be considered below) indicates increased permeability to ions. After the corpuscle becomes permeable to ions, further increase in conductivity might be due to liberation of ions from combinations with colloids in the interior. However many ions, for instance PO_4, cannot be liberated without incineration or other rigorous treatment. Increase in conductivity of the blood by laking agents has been proven to be chiefly due to increased permeability of the corpuscles, since the conductivity of the serum never shows so great an increase on the addition of the laking agent, and is usually diminished (by the hæmoglobin) if the corpuscles are present.

The portion of the normal corpuscle presenting the greatest resistance to the electric current is the surface layer, since Höber[3] observed that the conductivity of the interior of the corpuscle (determined by its dielectric value) is many times greater than that of the corpuscle as a whole. Peskind[4] caused bubbles of nitrogen to form within the corpuscle and observed that they were retained by a superficial membrane. This may be the membrane which resists the electric current.

The chemical composition of the corpuscle is supposed to bear some relation to its permeability. Aside from the hæmoglobin, and the rather low water content (60 per cent.) the corpuscle

[1] *Science*, Jan. 22, 1897.

[2] Moore and Roaf, *Biochem. Jour.*, III., 155.

[3] *Pflüger's Arch.*, 1910, CXXXIII., 237.

[4] *Am. Jour. Physiol.*, VIII.

is composed of lecithin and cholesterin with a little nucleoproteid. It is probable that these lipoids are chemically different in different species of animals, since Lefmann[1] observed that the lipoids of erythrocytes of the same species are not toxic, whereas those of another species may be very toxic.

The distribution of these substances in the corpuscle has not been ascertained. Pascucci[2] supposed the corpuscle to be a bag of proteid impregnated with lecithin and cholesterin and filled with hæmoglobin. He found that artificial lecithin-cholesterin membranes were made more permeable to hæmoglobin by the laking agents, saponin, solanin and tetanus or cobra poison. Dantwitz and Landsteiner suppose the lecithin to be in combination with protein.

Hoppe-Seyler assumed the hæmoglobin to be in combination with lecithin in the corpuscle, and Bang[3] has shown that lipoids may be fixed by hæmoglobin. It seems evident that there does not exist an aqueous solution of hæmoglobin within the corpuscle, since hæmoglobin crystals may be made to form in *Necturus* corpuscles without extraction of water. Furthermore, Traube and Goldenthal[4] find that hæmoglobin has a hæmolytic action, and unless there exists some body within the corpuscle which antagonizes this action (as serum does) a hæmoglobin solution could not be retained by the corpuscle. Probably all of the so-called "stroma" constituents, not in combination with the hæmoglobin, form the plasma membrane of the corpuscle.

Under certain conditions, the hæmoglobin comes out of the corpuscles, and the blood is said to be laked. Laking of "fixed" corpuscles occurs only after the removal of the fixing reagent. Thus, sublimate-fixed corpuscles may be laked by substances which combine with mercury, such as potassium iodide, sodium hyposulphite or even serum proteids. The fact that they may be laked by heating in water is probably because the nucleo-histone is not fixed by sublimate. This process is prevented by hypertonic NaCl solution, presumably on account of its power to precipitate nucleo-histone (Stewart). Formaldehyde-fixed corpuscles may

[1] *Beiträge chem. Physiol. u. Path.*, XI., 255.
[2] *Hofmeister's Beiträge*, 1905, VI., 543, 552.
[3] *Ergeb. d. Physiol.*, 1907, VI., 152.
[4] *Biochem. Zeit.*, 1908, X., 390.

be laked by ammoniacal water, at a temperature which must be higher, the more thoroughly they have been fixed. Ammonia combines with formaldehyde.

Stewart[1] supposes that the hæmoglobin must be liberated from some compound before the blood can be laked. We cannot say that the corpuscle is always permeable to hæmoglobin from within outward. However the corpuscle probably is impermeable to it from without inward, since it does not take up hæmoglobin from a solution, and after the blood is laked the serum contains hæmoglobin in greater concentration than the "ghosts" do.

At any rate, permeability to hæmoglobin appears to be independent of permeability to salts, since Rollett[2] found that laking by condenser discharges may set free the hæmoglobin without the corpuscle becoming permeable to ions. Stewart[3] concluded that the same is true of laking with sodium taurocholate (even after considering the depressing action of hæmoglobin on the conductivity).

Stewart[4] and others had already shown that blood laked by minimal applications of such laking agents as freezing and thawing, heating (to 60°), foreign serum, and autolysis (spontaneous laking) cause but a slight increase in the permeability to ions, whereas the continued application of some of these agents, or especially such violent reagents as distilled water and saponin, cause a marked increase in electric conductivity. On the other hand, if saponin is added to defibrinated blood at 0°, the conductivity of the corpuscles to ions begins to increase before any hæmoglobin escapes from the corpuscles.

The liberation of the hæmoglobin by some laking agents may be due to the direct action of the reagent in breaking up the compound in which the blood pigment exists, but is probably sometimes a secondary effect, following increase in permeability to electrolytes.

It has been shown that many laking agents, lipoid solvents, saponin unsaturated fatty acids, soaps, and hæmolysins (containing lipase) are such as would alter lipoids physically or

[1] *Jour. Pharm. and Exper. Therapeutics*, 1909, I., 49.

[2] *Pflüger's Arch.*, 1900, LXXXII., 199.

[3] *Am. Jour. Physiol.*, X.

[4] *Jour. Physiol.*, 1899, XXIV., 211.

chemically, whereas pressure, trituration, shaking, heat, condensor discharges, freezing and thawing, water, drying and moistening, salts (including bile salts), acids and alkalis, might act also on proteids.

Since any treatment which causes great swelling[1] of the corpuscle leads to loss of hæmoglobin, it is probable that stretching or breaking of the surface film increases its permeability. But laking may occur without swelling, and even crenated corpuscles may be laked by sodium taurocholate.

Höber[2] observed that the relative action of ions in favoring hæmolysis is: salicylate > benzoate > I > NO_3, Br > Cl > SO_4 and K > Rb > Cs > Na, Li. Since this is the order in which they affect the aggregation state of colloids, their action is probably on the aggregation state of the colloids of the corpuscle (proteids or lipoids or their combinations).

The permeability of formaldehyde-fixed corpuscles to ions, is greatly increased by extraction of the lipoids with ether, or by treatment with substances such as saponin, which act on lipoids. Since the proteids have been thoroughly fixed, it is evident that they play no part in this process, though they may do so in the non-fixed corpuscles.

The relation of lipoids outside of the corpuscles to hæmolysis has been extensively investigated, and cannot be fully treated here. Willstätter found that cholesterin combines with one of the saponins, destroying its hæmolytic power. Iscovesco[3] concludes that cholesterin combines with soap, and prevents its toxic action.

Changes in permeability of the corpuscles to ions were studied chemically before the application of the electrolytic method. Hamburger[4] and Limbeck[5] observed that when CO_2 is passed through blood, chlorine passes from serum into corpuscles and the alkalescence of the serum is increased. On the other hand, the distribution of sodium and potassium is not changed.[6]

[1] Roaf, *Q. J. Exper. Physiol.*, III., 75, supposes this swelling to be due to ionization and hence increased osmotic pressure of hæmoglobin.

[2] *Biochem. Zeit.*, 1908, XIV., 209, and *loc. cit.*

[3] *Comptes Rendus, Soc. Biol.*, 1910, LXIX., 566.

[4] *Zeit. f. Biol.*, 1891, XXVIII., 405.

[5] *Arch. exp. Path.*, 1895, XXXV., 309.

[6] Gürber, *Sitzungsber. physik.-med. Ges. Wurzburg*, 1895.

Koeppe[1] and Höber[2] explain this process in the following manner: The lipoid-soluble CO_2 enters the corpuscle, and by reacting with alkali albuminates in the protoplasm, gives off more anions than it does in the serum. During the presence of CO_2, the corpuscle is permeable to anions, and the $CO_3^=$ or HCO_3^- ions pass back into the serum, being exchanged for Cl^- ions to equalize the electrical potential. Sodium bicarbonate being more alkalescent than sodium chloride, the titratable alkalinity of the serum is increased.

This explanation is supported by the following facts: When CO_2 is passed through a suspension of erythrocytes in cane sugar solution the latter does not become alkaline. If CO_2 is passed through a mass of centrifuged erythocytes, which are then added to physiological salt solution, the latter becomes more alkaline than the serum in Hamburger's experiment. Any sodium salt may be substituted for serum, and its anions will pass into the corpuscles.[3] Also the number of ionic valences passing into the corpuscle is constant, *i. e.*, if sulphate is used only half as many ions enter the corpuscles as when chloride or nitrate is used. The process is reversed by removal of the CO_2.

This same phenomenon has been observed in leucocytes by van der Schroeff.

There seems to be some relation between hæmolysis and agglutination of the corpuscles. Arrhenius[4] supposed that agglutination by acids is due to the coagulation of the proteids of the envelope. However, since agglutination is followed by precipitation, it seems probable that the loss of the negative electric charge which tends to keep the corpuscle in suspension and causes it to repel every other corpuscle, is partly responsible for the phenomena.

The fact that water-laking is preceded by agglutination might be explained if we assume that increase in permeability to ions leads to loss of electric charge. The charge may be due to the charges on the colloids of the corpuscle or to semi-permeability to ions. The corpuscle is very poorly permeable to ions, but may

[1] *Pflüger's Arch.*, 1897, LXVII., 189.

[2] *Pflüger's Arch.*, 1904, CII., 196.

[3] Hamburger and van Lier, *Engelmann's Arch.*, 1902, 492.

[4] *Biochem. Zeit.*, 1907, VI., 358.

be slightly more permeable to some one ion than to others. If this ion were more concentrated in the plasma or in the corpuscle, the latter would become electrically charged, and a general increase in ionic permeability would lead to a reduction or loss of this charge. The loss of charge would favor their coming in contact with one another and their precipitation, but their cohesion is probably due to some other change, possibly the exit of adhesive substances, on increase in permeability.

VI. Absorption and Secretion.

1. *Absorption through the Gut.*

If a live vertebrate intestine be filled with one portion of a physiological NaCl solution, and suspended in another portion of the same solution, fluid will pass through the wall of the gut from within outward. Cohnheim[1] found that holothurian gut behaves in the same way toward sea water, and the absorption stops if the gut is injured with chloroform or sodium fluoride.

It might be supposed that the hydrostatic pressure produced by the contraction of the musculature, is the driving force of absorption, but on the contrary, Reid[2] found that the wall of the rabbit's intestine behaved in the same way when used as a diaphragm.

Salt is absorbed by an intestine filled with a very hypotonic solution of it, and water may be absorbed when the solution is very hypertonic.

Blood salts enter the intestine when it is injured by an extremely hypertonic solution, or sodium fluoride, chinin or arsenic.

Grape sugar and sodium iodide may pass from without inwards through the wall of a normal holothurian intestine.

Traube[3] claims that absorption is explained by his observation that the surface tension of the contents of the gut is less than that of the blood, but this does not apply to the experiments in which an identical solution was placed on each surface of the wall of the gut. Traube[4] found that the addition of a substance

[1] *Zeit. physiol. Chem.*, 1901, XXXIII., 9.

[2] *Jour. Physiol.*, 1901, XXVI., 436.

[3] *Pflüger's Arch.*, 1904, CV., 559. Cf. Iscovesco, *Comptes Rendus, Soc. Biol.*, 1911, LXXI., 637.

[4] *Biochem. Zeit.*, 1910, XXIV., 323.

lowering the surface tension increased the absorption of NaCl by the gut.

Absorption is probably due to irreciprocal permeability of the wall of the gut. Hamburger showed that dead gut and even artificial membranes showed irreciprocal permeability to certain substances. These artificial membranes were of different composition on their opposite surfaces (parchment paper-chrome albumin, or parchment paper-collodion) and he assumed that the wall of the gut is composed of two osmotically different layers. In reality there may be more than two such layers, and the plasma membranes of the individual cells of the gut may show irreciprocal permeability.

Traube[1] showed that the rate of absorption of a substance by living gut is usually greater the more it lowers the surface tension of water. The order of ions is: $Cl > Br > I > NO_3 > SO_4$, HPO_4 and K, Na > Ca, Mg. The order of non-electrolytes, according to Katzenellenbogen[2] is: glycocoll < urea < acetone, mannit < erythrite < glycerine < acetamid, methylalcohol, propylalcohol, amylalcohol.

The rate of absorption through dead ox gut according to Hedin[3] is: $Br > NO_3 > Cl > SO_4$ and K > Rb > Na > Li > Mg and mannit < erythrite < glycerine < urethan < glycocoll < amylenhydrate < glycol < urea < propylalcohol < isobutylalcohol < methylalcohol, ethylalcohol.

The action of poisons on absorption may be due to the alteration of the plasma membranes of the individual cells. Mayerhofer and Stein[4] state that even sugar in certain concentrations increased the permeability of the gut.

2. *Osmotic Relation of Aquatic Animals to the Medium.*

Fredericq found that the salt content of the body fluids of marine invertebrates is about the same as that of sea water. Henri and Lalou[5] showed that the osmotic exchange between cœlom fluid of sea urchins and holothurians and medium is chiefly

[1] *Pflüger's Arch.*, CXXXII.
[2] *Pflüger's Arch.*, CXIV., 522.
[3] *Pflüger's Arch.*, 1899, LXXVIII., 205.
[4] *Biochem. Zeit.*, 1910, XXVII., 376.
[5] Winterstein, II. (2), 2.

water. If the sea water was diluted with ¼ vol. of isotonic cane sugar solution, the salt content of the cœlom fluid is very little lowered in 4 hours, and only traces of sugar appear in it. The result is the same with isotonic urea (which easily penetrates most plasma membranes). But the salt content of the blood of elasmobranchs and teleosts is about half that of the sea.

Botazzi and his colleagues observed that the osmotic pressure of the blood of elasmobranchs is about equal to that of the medium, the salts in the blood being supplemented by organic substances, chiefly urea, of which there is 2–3 per cent.

If elasmobranchs are placed in concentrated sea water, the osmotic pressure of the blood rises, but the ratio of urea to salts remains the same. G. G. Scott found that changes in the density and osmotic pressure of the blood of elasmobranchs accompany changes in the salt content of the medium.

However, in marine teleosts as well as all fresh-water animals which have been studied in this respect, both salinity and osmotic pressure of the body fluids are very different from that of the medium.

The osmotic pressure of the blood of marine teleosts is about half that of the sea, but in fresh-water teleosts it is still less (but much greater than the fresh water). This indicates that there must be a change in the osmotic pressure of the blood as the fish ascends a river. Greene[1] observed that it took salmon 30–40 days to pass the brackish water, in which time they were acclimatized to fresh water. After being in fresh water 8–12 weeks, the osmotic pressure of the blood was reduced only 17.6 per cent. This reduction may be partly accounted for by the absorption of the osmotic substances in the blood by the sexual glands. In harmony with this view is the fact that the osmotic pressure of the blood of the female was reduced much more than that of the male. One salmon, that was very weak and probably dying, showed 32 per cent. decrease in Δ of blood. Sumner[2] observed that changes in weight and salt content of marine teleosts accompany, but are not proportional to changes in the medium.

[1] U. S. B. F., 1904, XXIV., 445; 1909, XXIX., 129; *Jour. Exp. Zoöl.*, 1910, IX.

[2] Bull. U. S. B. F., 1905, XXV., 53, and *Am. Jour. Physiol.*, 1907, XIX., 61.

Overton observed that if the cloaca and mouth of a frog in fresh water are closed, the frog constantly increases in weight. This can be prevented by the addition of .7 per cent. NaCl to the medium. In a hypotonic solution water is constantly absorbed by the skin and excreted by the kidneys. Fischer's[1] experiment, in which ligature of the leg of a frog caused great swelling below the ligature is probably to be explained by the fact that water was absorbed by the skin but could not reach the kidneys, since the blood circulation was stopped. In regard to Fischer's explanation, compare the results of Sidbury and Gies.[2] Sumner concluded that in the fish, the gills are the chief seat of osmotic exchange.

It appears, therefore, that osmosis occurs through the integument (including gills), kidneys and gut simultaneously, and since the contents of the gut and kidney tubules are not the same as the medium, we should not expect an osmotic equilibrium between the body fluids and the medium. Furthermore, all three of these membranes may show irreciprocal permeability.

Fresh-water fish and non-migratory marine fish are killed by great changes in the medium, even though it be very gradual.

Bert maintained that if fresh-water fish are placed in sea water, the gill capillaries contract and become blocked by the distorted corpuscles. In naked-skinned fishes, not only the gills are affected, but water may be lost from the tissues.

Bert and Sumner both agree that the salts in sea water cannot be replaced by any other substance, without causing the death of certain marine fishes. Mosso[3] claimed that when sharks are placed in fresh water, the gill capillaries become so blocked with laked corpuscles that physiological salt solution could not be forced through them. He observed that the differences in the resistance of certain fish to changes in the salt content of the medium, corresponded to differences in the resistance of their blood cells to the hæmolytic action of such changes. Sumner,[4] however, states that this blocking of gill capillaries does not occur in sharks or marine teleosts in fresh water.

[1] Fischer, M. H., "Œdema," J. Wiley & Sons, 1910.

[2] *Soc. Exper. Bio. and Medicine*, 1911, VII., 104.

[3] *Biol. Centlb.*, 1890, X., 570.

[4] *Proc. Seventh Internat. Zoöl. Congress*, Boston, 1907.

Sumner showed that as the fish becomes enfeebled by the abnormal medium, it becomes more permeable to salts.[1] Whether the direct action of the abnormal medium, or the blocking of the gill capillaries, produce the increase in permeability, has not been experimentally tested. However, the gills themselves would not be *asphyxiated* by blocking of their capillaries, and it seems probable that the change in permeability is due to the direct action of the medium.

We may conclude therefore that the death of the fish results from the osmotic exchange. This may be sufficient to cause death while the fish still maintains its normal semi-permeability, or death may occur only after increase in permeability, due to the direct action of the medium on the osmotic membranes.

A similar increase in permeability may explain Wo. Ostwald's observations on fresh-water *Gammarus* in pure salt solutions.[2] He found that the ratio of the rapidity of death to the concentration is about constant up to a certain point, above which it is much greater. This critical concentration has nothing to do with the osmotic pressure, since it is different for different salts. Perhaps at this concentration the salt made the membranes more permeable.

Schücking[3] found that nicotine and strychnine made the skin of *Aplysia* more permeable to salts. Since cocain retarded shrinkage in hypertonic solution, he supposed that the hydrostatic pressure produced by the muscles aided shrinkage. However the hydrostatic pressure is probably very small, and the effect might have been due chiefly to an increase in permeability to salts, produced by the cocain.

3. *Secretion of Lymph and Tissue Juice.*

Höber supposes the raising of the osmotic pressure by the katabolism of the tissues, causes fluid to be drawn out of the blood-vessels, and states that the lymph in the thoracic duct has a greater osmotic pressure than the blood.

Traube states that the surface tension of transudates and

[1] Cf. Greene, above.

[2] *Pflüger's Arch.*, 1905, CVI., 568.

[3] *Arch. Anat. Physiol.*, Physiol. Abt., 1902, 533.

exudates is always greater than that of the blood. He cites a case in which a transudate was caused to be absorbed by injecting into it a substance which decreased its surface tension.

4. *Excretion.*

Milk and bile have about the same osmotic pressure as the blood, but urine is almost dry in some animals; it is usually hypertonic in man but may be hypotonic.

Traube maintains that the surface tension of the normal urine is always greater than that of the blood, and that this is the driving force in excretion.

However, Höber and others suppose that the substances to be excreted may be formed into solid bodies in the tubule cells, and thrown out into the lumen.

If lipoid-insoluble dyes are fed to frogs, granules in the cells of certain segments of the kidney tubule are stained with them. The dye is not first excreted by the glomeruli and then absorbed from the lumen by the tubule cells, for if the vena Jacobsoni, which supplies the tubules, is ligatured, no staining occurs, although the renal arteries still supply the glomeruli.

The stained granules in the tubule cells are thrown out into the lumen and pass into the bladder. These granules usually dissolve to form a slimy substance in the urine, but some of them may remain intact.

The circulation in mammalian kidneys cannot be controlled in the same way, but after intravenous injection of a certain lipoid-insoluble dye, no stain may be detected in the walls of the glomeruli, although the tubule cells are stained. The stain in the lumen does not appear above the level of the stained tubule cells. In the excretion of carmine, it may be found in granules in the tubule cells and lumen, similar to those found in frog's kidneys.

It has been supposed that urea is excreted by collecting in these granules and passing out with them, but it would be even simpler to assume that some substance is excreted into the lumen, which combines with urea and so lowers the concentration of that in solution, thus accelerating its excretion.

The chief recommendation for the granules is their valve-like

action, which would account for the secretion of urine against a concentration gradient, but a simpler mechanism of such a process is shown in Hamburger's double membranes.

The blood pressure may aid in the secretion of the water of the urine, which is eliminated chiefly through the glomeruli, but its insignificance in the elimination of urea is shown by the fact that after increasing the volume (and therefore pressure) of rabbit's blood 70 per cent. by transfusion, the urea elimination was not or only very slightly increased.

VII. Cell Division.

Various hypotheses as to the cause of cell division have been advanced by the morphologists. Hertwig, supposed that when the ratio of nucleus to cytoplasm is less than normal, the cell will divide.[1] Gerassimow[2] subjected cells of *Spirogyra* to low temperatures and other abnormal conditions and obtained an increased amount of chromatin in some of them. These cells did not divide until the ratio of nucleus to cytoplasm was as great as at the time of division of a normal cell.

I found that chromatin is not necessary for cell division.[3] After extracting the chromosomes from the starfish egg, I caused it to divide. In this case the ratio of nucleus to cytoplasm was zero; however the cell did not continue to divide indefinitely.

There is no easy method of determining the ratio of nucleus to cytoplasm. Some cells contain large vacuoles whose contents are not considered as cytoplasm. Eggs contain fat drops and granules compounded of protein and lipoids. These are not considered as cytoplasm by all investigators. If the granules and oil are included as cytoplasm, the ratio of nucleus to cytoplasm is very small, and yet the egg cell does not divide unless "stimulated" by the sperm or some other means.

R. Lillie[4] observed that chemical substances, which in low concentration cause the *Arbacia* egg to divide, in high concentration cause outward diffusion of the red pigment (echinochrome) and compared this to the laking of erythrocytes.

[1] He is not confirmed by Conklin, *Jour. Exper. Zoöl.*, 1912, XII., 1.

[2] *Bull. Soc. Imp. Nat.*, Moskau, 1904, No. 1.

[3] McClendon, *Arch. f. Entwicklungsmech.*, 1908, XXVI, 662.

[4] Biol. Bull., 1909, XVII., 188.

This is made more striking by the fact, mentioned first by Loeb, that hæmolytic agents are effective in artificial parthenogenesis. R. Lillie observed that pure solutions of sodium salts caused the egg to divide, the order of effectiveness of anions being $Cl < Br < ClO_3 < NO_3 < CNS < I$. He also found that these salts could be inhibited by others ($CaCl_2$, $MgCl_2$), as is characteristic of the antagonistic effects of salts in physiological phenomena, and the precipitation of colloids.

I found that the sea urchin's egg contains fatty substances, and relatively large amounts of lecithin probably in combination with proteids. I found that *Toxopneustes* eggs freed from the jelly-like coverings, contained about 10 per cent. lecithin (alcohol extract ppt. with acetone) and about 2 per cent. of an extract soluble in alcohol or acetone and containing rosettes of fat-like crystals. This extract blackened strongly with osmic tetroxide and effervesced on adding dry Na-carbonate in water, then emulsified, probably it contained unsaturated fatty acid.

According to a private communication by Mathews, the egg of the starfish contains lecithin and an unsaturated fatty acid, but no cholesterin. In this last characteristic it differs markedly from the erythrocyte. There is no way of determining whether these substances enter into the composition of the plasma membrane, but the facts are presented in order to indicate the possibilities.

We have seen that the exit of hæmoglobin is probably not due to increased permeability to this substance. It is possible that the same is true of echinochrome. I found that the echinochrome in the egg shows a continuous spectrum, whereas that extracted in various ways shows characteristic bands. It may possibly be held by chemical combination in the egg.

However I found other evidence for increase in permeability of the sea urchin's egg coincident with beginning development:[1]

1. Fertilized eggs are caused to shrink more quickly than unfertilized eggs, with isotonic sugar solution. Presumably the fertilized eggs are more permeable to the substances exerting the internal osmotic pressure.

2. The electric conductivity of the egg increases about ¼ when

[1] McClendon, *Amer. Jour. Physiol.*, 1910, XXVII., 240.

it is fertilized or made parthenogenetic with acetic acid, indicating increased permeability to ions.

Lyon and Shackell[1] and Harvey[2] observed that methylene blue and neutral red enter fertilized eggs more quickly than unfertilized eggs. Harvey supposed that only the free color base (undissociated) entered, since the addition of a little acid to the sea water prevented the staining of the eggs.

Mathews[3] considered the penetration of stains into the egg as a chemical process (the stain forming a salt combination with the lecithin or proteins of the egg surface).

Harvey observed, further, that NaOH penetrates fertilized more easily than unfertilized eggs, but the eggs are killed by the alkali.

The fact that the unfertilized frog's egg continues to swell *for a long time* in water (Biataszewitz) whereas the osmotic pressure of the fertilized frog's egg is *quickly* reduced to equal that of the medium (Backmann and Runnström) indicates increase in permeability to osmotic substances on fertilization. In this connection it is interesting to note that Bataillon,[4] Brachet, and myself[5] caused the unfertilized frog's egg to rotate normally and segment merely by pricking it.

It has been supposed by various observers that the "formation" of the fertilization membrane in very closely related to the segmentation of the egg. Loeb observed that the sea urchin's egg may develop without the formation of a fertilization membrane, and I have confirmed this observation, and shown that it is very probably wrong to suppose that this is a case of failure in "pushing out" of the membrane. Apparently "membrane formation" is not essential for the segmentation of the egg, although by furnishing protection it may insure the development of the embryo.

Loeb postulated that an osmotically active colloid exists in the unfertilized egg, but is so covered with lipoids that it does not absorb water until it is squeezed out or otherwise exposed

[1] *Science*, 1910, XXXII., 250.

[2] *Ibid.*, p. 565.

[3] *Jour. Pharmacol. and Exp. Ther.*, 1910, II., 201.

[4] *Arch. Zool. Expér.*, 1910 (5), VI., 101.

[5] McClendon, *Amer. Jour. Physiol.*, 1912, XXIX., 298.

at the surface of the egg, at the beginning of development (when it fills the so-called "perivitelline space"). I observed that this substance bears a positive charge (is basic) since it migrates toward the kathode when an electric current is passed through sea water containing the fertilized egg.

The unfertilized egg is imbedded in a mass of jelly which is probably mucin. This jelly bears a negative charge (is acid) since it combines with color bases.

When the positively charged colloid is exposed at the surface (on increase in permeability) and comes in contact with the negatively charged jelly, the two mutually precipitate at their surface of contact, thus forming the fertilization membrane. But if all of the jelly is washed off of the egg before the latter is caused to develop, no fertilization membrane is formed (as I have observed) because no two oppositely charged colloids are brought in contact, but the basic colloid may with difficulty be seen as a refractive layer, which has been mistaken for a poorly developed "fertilization membrane."

The observation of Lyon[1] makes it appear that catalase comes out of fertilized more quickly than unfertilized eggs, probably due to increased permeability.

Lyon observed that CO_2 came out of fertilized more quickly than unfertilized eggs, and O. Warburg, Loeb and myself[2] observed that oxygen is absorbed more rapidly by the former. We might ask: Does increased permeability allow increased oxidation, or is increased oxidation the primary cause of the increased respiration?

The permeability change is the simplest explanation, and in what other way could oxidation be increased? Loeb supposed the sperm carried an oxidase into the egg.[3] But no addition of oxidase is concerned in artificial parthenogenesis, and Loeb assumed that the oxidase (or other enzyme, kinase?) is held in the egg periphery and cannot penetrate the egg interior until the permeability is increased.

In addition to oxygen, oxidase, and escape of CO_2, hydroxyl

[1] *Am. Jour. Physiol.*, 1909, IV., 199.

[2] McClendon and Mitchell, *Jour. Biol. Chem.*, 1912, X., 459.

[3] In this connection it is interesting to note that Masing, *Zeit. physiol. Chem.*, 1910, LXVI., 265, failed to find more iron in sperm than in sea water.

ions are necessary for the rapid oxidation of the sea urchin egg (Loeb), and Harvey showed that the unfertilized egg is practically impermeable to OH ions of low concentration. The increased permeability allows hydroxyl ions in the sea water to penetrate the egg, as shown by Harvey, and, since the sea is always alkaline, this may explain the increased oxidation.

Asters always develop in the egg before segmentation. In the normal egg these have some relation to the division of the nucleus, but even if a nucleus is not present, I have observed that the cytoplasm constricts along a line on the surface farthest removed from the centers of the asters.

The constriction of the cytoplasm is probably due to a band of increased surface tension (or to decreased surface tension at the poles). This might be caused by local increase in permeability to ions, causing decreased polarization, at the equator (or increased polarization at the poles, due to increased production of the polarizing electrolyte in the asters).

The same reasons that were given for assuming that the surface of the *Amœba* is electrically polarized, hold good for the egg. The first change is probably a general increase in surface tension, indicated by rounding up of the egg. Later this may become localized from internal causes and result in cleavage.

Hyde[1] observed local changes in electric polarization of *Fundulus* eggs during cleavage, indicating that surface tension changes and cleavage are due to this cause.

It has been objected that the segmentation of the egg is not a typical case of cell division, since the egg cell is "wound up" and ready for some "stimulus" to set it going, whereas tissue cells must "grow" or "rest" after each division before dividing again.

It may be true that growth is prerequisite to division, but this cannot be formulated quantitatively. In the spore-formation of certain organisms, a cell may divide in a relatively short time into myriads of almost ultra-microscopic cells.

Hertwig may be right, in general, in assuming that the relative growth of nucleus and cytoplasm influences division, but the difficulties in proving this have been indicated, and this cannot

[1] *Am. Jour. Physiol.*, XII., 241.

be expressed in chemical terms. It is generally supposed that nucleic acid is a more abundant constituent of the nucleus than of the cytoplasm, but much evidence has appeared for believing that it is often present in considerable quantities in the cytoplasm. Loeb supposed that the segmentation of the sea urchin egg is accompanied by an "autocatalytic" synthesis of nucleic acid, since the nuclei increased in number. But Masing[1] and more recently Shackell[2] by chemical analysis found as much nucleic acid in the unsegmented egg or 1-cell stage as in the blastula stage.

There is some indirect evidence that increase in permeability may cause an increased division rate of tissue cells. Though cell growth may influence division, it is probable that permeability influences growth.

Various "stimuli" cause increased proliferation of cells of the germinal layer of the skin. It is commonly known that mechanical stimuli increase growth of the skin.

Bernhard Fisher observed that Sudan III. or Scharlack R[3] cause increased proliferation of the epidermis. When the dye is injected under the skin of a rabbit the skin grows toward the dye.

Furst[4] found that gradual increase of temperature caused a corresponding increase in proliferation of tissue cells (due to increased chemical reaction and inflammation of the tissue). But when a certain temperature was reached a sudden jump in the increase in proliferation was observed without a corresponding increase in inflammation. This is similar to the phenomenon seen in unfertilized eggs, where a rise in temperature beyond a certain point causes segmentation.

It has also been observed that electrical stimulation may cause increased proliferation of tissue cells.

All of these changes (electrical, thermal, or mechanical stimulation, or treatment with lipoid soluble substances) cause in-

[1] *Zeit. physiol. Chem.*, 1910, LXXVII., 161.

[2] *Science*, 1911, n. s., XXXIV., 573.

[3] Which are practically insoluble in water but soluble in fats and lipoids and, as I have observed, slightly in lipoid-protein combinations.

[4] See v. Dungern u. Werner, "Das Wesen Bösartigen Geschwülste," Leipzig, 1907, p. 65.

creased permeability and segmentation of the sea urchin's egg. Therefore, from analogy, we may conclude that increase in permeability may cause tissue cells to divide.

The "wound stimulus" to regeneration of tissue may also cause increased permeability of the cells.

In a preceding chapter it was shown that the "current of injury" produced by the negative electric potential of a wounded surface is common to animal and plant tissues. The wounded cell acts as an electric generator and a current flows through neighboring cells.

I observed that if a current is passed through living tissue, which is subsequently fixed and stained, basophile substances will be found displaced toward the anode. In sections of tissue adjacent to a wound the extent of the current is indicated by the displacement of basophile granules. The current affects first the cells in contact with the wounded cells, then extends in some directions more than others. Electric currents ("currents of growth") continue for many days after the wound has healed.

Since electric currents cause sea-urchin eggs and tissue cells to divide and proliferate, probably these bio-electric currents constitute the so-called "formative stimulus" of regeneration.

Embryonic cells, cells of germinal regions, and cancer cells are distinguished by their great power of proliferation, or rapid division. It is probable that the plasma membranes of these cells are more permeable than those of other tissue cells in the same medium or under the same conditions.

Cancers have been produced by the action of X-rays (electric pulsations) on the skin. The cells in the skin were so changed that they proliferated more rapidly. Similarly, electric changes have been observed to start the egg cell to rapid proliferation. There is probably some irreversible change in the permeability of these cells, which does not, however, make the plasma membrane incapable of subsequent reversible changes in permeability (*i. e.*, the change is unlike what occurs at death of the cell).

The suggestion that cancer cells are more permeable than tissue cells in general may possibly be of therapeutic importance. Loeb has shown that fertilized eggs are more sensitive than unfertilized eggs to various toxic substances (probably because

these substances enter the fertilized eggs more easily). The same explanation may possibly be applied to the effect of sugar on certain living cells. The unfertilized eggs of the frog, *petromyzon*, sea urchin and annelid have been caused to segment, by placing them in sugar solutions. Mayerhofer and Stein[1] observed that sugar in certain concentrations increased the permeability of the gut to certain salts, and in this condition the gut was more easily injured by the diffusion of substances.

Similarly Stockard observed that sugar increased the toxicity of pure solutions of salts on the *Fundulus* egg. Morgan and Stockard[2] showed that this was not due to the inversion of sugar or to the osmotic pressure, and supposed that the sugar might combine chemically with the salt. It seems probable that the sugar increased the permeability to salt. The fact that sugar in fresh water is toxic whereas the same amount of sugar in the normal medium (sea water) is not toxic or less toxic, indicates that the salts within the *Fundulus* egg are the same as those outside (in sea water), and increase in permeability to them does not lead to diffusion while they remain in sea water, but diffusion takes place in fresh water.[3]

If it be shown that cancer cells are more permeable, substances may be found which kill cancer cells more easily than tissue cells as explained below.

Whereas a certain increase in permeability of the cell seems to cause division, a very great increase in permeability causes death (hæmolysis, cytolysis, bacteriolysis). It has been shown that certain lysins are specific for certain cells, probably because the plasma membranes of these cells differ chemically.

The fertilized egg is more easily cytolyzed than the unfertilized egg by certain substances. It therefore appears that the more permeable the cell is in the beginning, the more easily is the permeability brought to the point which causes cytolysis.

Hence it is probable that certain substances may be found by which cancer cells can be more easily cytolyzed than normal tissue cells.

[1] *Biochem. Zeit.*, 1910, XXVII., 376.

[2] BIOL. BULL., 1907, XIII., 272.

[3] In the absence of sugar I have shown that no diffusion takes place in fresh water. *Amer. Jour. Physiol.*, 1912, XXIX., 295.

It has been shown that narcosis is accompanied by decreased permeability. On the other hand, certain forms of inhibition of muscle are accompanied by an increase in permeability. May certain cells be inhibited in proliferation by an increase in permeability, too great for cell division but not great enough for cytolysis? The great oxidation rate in eggs inhibited in cleavage by very hypertonic solutions as determined by Warburg, seem to indicate this.

It has been shown that certain tissue cells inhibit the proliferation of others. In the healing of wounds, the epidermis inhibits the growth of connective tissue. If a wound remains uncovered by epidermis for a relatively long time, processes of connective tissue may grow outward, but this is prevented by the growth or transplantation of epidermis over the wound.

Perhaps the proliferation of the connective tissue is due to abnormal "stimuli" (bio-electric currents, diffusion of substances) such as cause proliferation in regenerating tissue generally. The presence of epidermis over the wound might protect the connective tissue from these "stimuli."

The foregoing facts and the speculations based on them may not be of far-reaching importance in themselves, but they suggest lines of research, which if followed, it is hoped, will add a great deal to cell physiology and pathology and be an aid to the understanding of many problems in therapeutics.